ab176764

Cellular Membrane Potential Assay Kit (Fluorometric-orange)

Instructions for Use

For the detection of membrane potential change in cells.

This product is for research use only and is not intended for diagnostic use.

Version: 1 Last Updated: 6 February 2019

Table of Contents

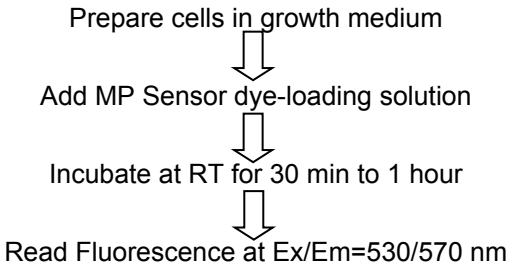
Table of Contents		2
1.	Overview	3
2.	Protocol Summary	4
3.	Kit Components	4
4.	Storage and Stability	5
5.	Materials required, Not supplied	6
6.	Assay Protocol	6
7.	Data Analysis	9
8.	Troubleshooting	11

1. Overview

Membrane potential is the difference in voltage between the interior and exterior of a cell. The membrane potential allows a cell to function as a battery, providing power to operate a variety of "molecular devices" embedded in the membrane. In electrically excitable cells such as neurons, membrane potential is used for transmitting signals between different parts of a cell. Opening or closing of ion channels at one point in the membrane produces a local change in the membrane potential, which causes electric current to flow rapidly to other points in the membrane. Ion channels have been identified as important drug discovery targets.

Abcam's Cellular Membrane Potential Assay Kit (Fluorometric - Orange) (ab176764) is a homogeneous assay with fast read time. It uses a proprietary long wavelength membrane potential indicator to detect the membrane potential change that is caused by the opening and closing of the ion channels. The red fluorescence of the membrane potential indicator used in the kit has enhanced fluorescence upon entering cells and minimizes the interferences resulted from the screening compounds and/or cellular autofluorescence.

2. Protocol Summary



3. Kit Components

Item	1 x 96 tests	10 x 96 tests	100 x 96 tests	Storage upon arrival	Storage after use/ reconstitution
MP Sensor	15 µL	150 µL	10 x 150 µL	-20°C	-20°C
10X Assay Buffer	1 mL	10 mL	100 mL	-20°C	4°C
HHBS	9 mL	100 mL	N/A	-20°C	4°C

4. Storage and Stability

Upon arrival, store the kit at -20°C and protected from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm all buffers to room temperature before use.

5. Materials Required, Not Supplied

- 96 or 384-well poly-D lysine plates
- Centrifuge
- Cell incubator
- Multi-well spectrophotometer (ELISA reader)

6. Assay Protocol

1. Reagent Preparation

a) MP sensor dye-loading solution (for 1 plate):

Thaw 1 vial each of MP sensor, 10X Assay Buffer and HHBS at room temperature before use.

Note 1: 15 µL of MP sensor is enough for 1 plate, un-used MP sensor can be aliquoted and stored at <-20 °C for more than 6 months if the tubes are sealed tightly, avoiding light and repeated freeze-thaw cycles.

Note 2: 10X Assay Buffer and HHBS can be stored at 4°C for convenience.

b) 1X Assay Buffer:

Add 1 mL 10X Assay Buffer to 9 mL of HHBS (not included in the 100 plate kit) mix them well.

Note: 10 mL 1X Assay Buffer is enough for 1 plate, aliquot and store un-used 1X Assay Buffer at < -20 °C, avoid light and repeated freeze-thaw cycles.

c) MP sensor dye-loading solution for one cell plate:

Add 15 µL MP Sensor into 10mL of 1X Assay Buffer (from 1b) mixing them well. This working solution is stable for at least 2 hours at room temperature.

2. Sample Preparation

2.1 Adherent cells:

Plate cells overnight in growth medium at 40,000 to 80,000 cells/well/100µL for 96-well or 10,000 to 20,000 cells/well/25µL for 384-well plates.

2.2 Non-Adherent cells:

Centrifuge the cells from the culture medium and then suspend the cell pellets in equal amount of HHBS and MP Sensor at 125,000 to 250,000 cells/well/100µL for 96- well or 30,000 to 60,000 cells/well/25µL for 384-well poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with break off prior to the experiments.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for the membrane potential change.

3. Run Membrane Potential Assay

a) Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) MP dye-loading solution into the cell plate.

Note1: If your screening compounds interfere with growth medium and serum factors, then replace the growth medium with equal volume of HHBS buffer before adding the MP Sensor dye-loading buffer. Alternatively, cells can be grown in serum-free conditions.

Note2: Do NOT wash the cells after dye loading.

b) Incubate the dye-loading plate in a 5% CO_2 incubator for 30 minutes.

Note: In some cases, incubation at room temperature for 30 to 60 min may work better.

c) Prepare the compound plates by using HHBS or your desired buffer.

d) Run the membrane potential assay by monitoring the fluorescence at Ex/Em = 530/570 nm.

Note: It is important to run the signal test before your experiment. Different instruments have their own intensity range. Adjust the signal test intensity to the level of 10% to 15% of the maximum instrument intensity counts. For example, the maximum fluorescence intensity count for FLIPR-384 is 65,000, so the instrument settings should be adjusted to have its signal test intensity around 7,000 to 10,000.

7. Data Analysis

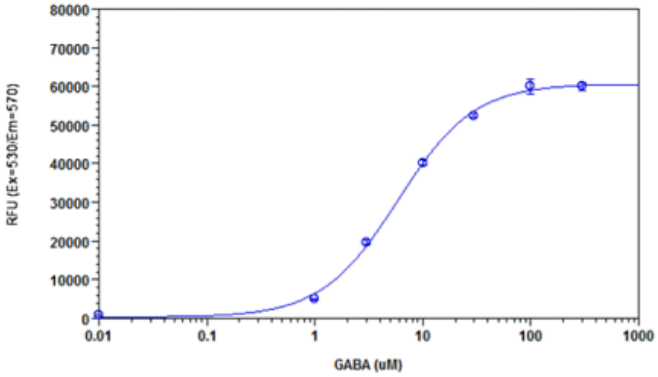


Figure 1. GABA Dose Response in WSS-1 cells measured with Abcam's Cellular Membrane Potential Assay Kit (Fluorometric - Orange) (ab176764).

WSS-1 cells were seeded overnight in 50,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate. The cells were incubated with 100 µL of Abcam's Cellular Membrane Potential Assay Kit (Fluorometric - Orange) (ab176764) for 30 minutes at room temperature. GABA (50µL/well) was added to achieve the final indicated concentrations.

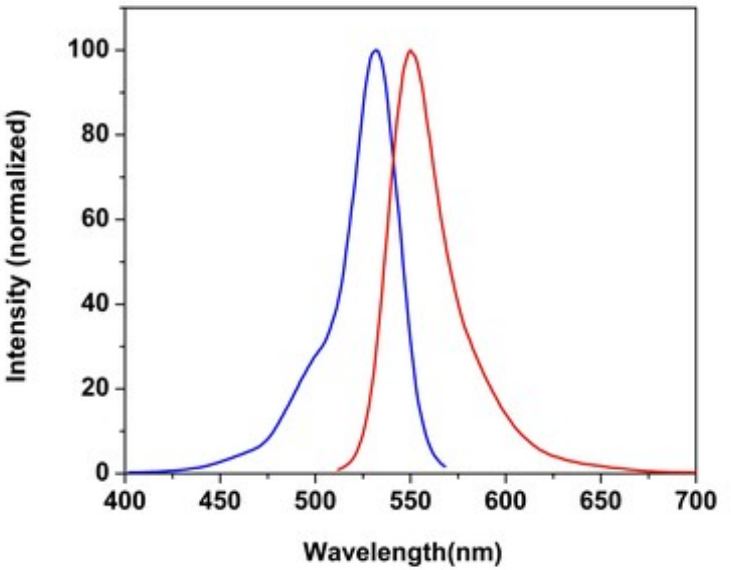


Figure 2. Excitation and Emission Spectra for Cellular Membrane Potential Assay Kit (Fluorometric - Orange) (ab176764).

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp